

FROM THE MEDICAL CLINIC OF THE UNIVERSITY OF ZÜRICH

Director: Professor Dr. Wilhelm Löffler

Work under the Direction of P.-D. Dr. S. Moeschlin

A Comparision of the Therapeutic Value of BAL and Sodium Citrate in Experimental Lead Poisoning

INAUGURALDISSERTATION

for the Degree of Doctor of Medicine
from the Medical Faculty of the
University of Zürich

Presented by

Leon Schechterman
of New York, U. S. A.

Genehmigt auf Antrag von Prof. Dr. med. Wilh. Löffler und
P.D. Dr. med. S. Moeschlin

Zürich 1953
Buchdruckerei Fluntern
Plattenstrasse 27

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CONTENTS

I. Introduction	7
II. Experimental Study	7
a) Materials and Methods	7
b) Experimental Results	8
III. Discussion and Conclusions	13
IV. Summary	17
V. Bibliography	18



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DEDICATED
TO MY DEAR PARENTS

I. INTRODUCTION

Although the incidence of lead poisoning has declined in the last decenium, plumbism still remains today, one of our most common industrial diseases. With the introduction of sodium citrate into the therapy of plumbism by Kety and Letonoff in 1943, a successful step forward was made in the treatment of this disease. The therapeutic value of sodium citrate has not yet been reported in animal experiments. BAL (British Anti-Lewisite), which was introduced by the British during the last war as an antidote for the war gas Lewisite, and then applied successfully clinically, as an effective antidote in arsenic and other heavy metal poisoning, has proved disappointing in experimental and clinical trials as a therapy for lead poisoning. Nevertheless, the remarkable increase in lead excretion produced by BAL, has caused speculation as to its role in the treatment of plumbism. These considerations led us to a comparative estimate of the therapeutic values of BAL and sodium citrate in experimentally induced lead poisoning.

II. EXPERIMENTAL STUDY

Materials and Methods

A series of sixty guinea pigs, ranging in weight from 300 to 680 grams, received in divided doses, through intraperitoneal injection, a total of 240 mgr. per kilogram body weight of a 3% sterile lead nitrate solution. The lead solution was injected over a period of 8 days according to the following plan: 1st day 120 mgr./kgr., 5th day 60 mgr./kgr., 8th day 60 mgr./kgr., for a total of 240 mgr./kgr. This injection schedule was arrived at in preliminary experiments, where an effort was made to determine a lead dosage that would be lethal for the guinea pigs, yet at the same time provide a reasonable period of time for the application of therapeutic measures. The aforementioned plan was found the most suitable and an experimental lead poisoning was thus produced of the sub-acute type. The intra-peritoneal injection route was chosen as the most rapid, accurate means of introducing lead into the animal system.

For the application of therapy the animals were divided into three groups of 20 animals each as follows:

Group I: 20 control animals.

Group II: 20 animals treated with a 5% sterile BAL solution (5% solution BAL in Arachnus oil + 10% Benzyl Benzoate) immediately following the initial lead injection. BAL dosage per injection was 3

mgr./kgr. body weight and applied as follows: 1, 2, 3, and 4 days — 4 times daily .06 ccm./kgr.. (5% BAL solution); from the 5—14 day—twice daily .06 ccm./kgr., sub-cutaneously injected.

Group III: 20 animals treated with a 5% sodium citrate solution immediately following the initial lead injection. The citrate solution was introduced daily into the animals by means of a stomach catheter. The daily dosage was 2×0.2 gm./kgr. body weight, that is 2×4 ccm./kgr. (5% sodium citrate solution) from the 1st to the 14th day; from the 15th to the 21st day only 4 ccm./kgr. daily.

All the above animals were maintained on the same diet. The animals were observed over a period of 4 weeks for weight loss, loss of appetite, and for characteristic changes in the blood picture. The initial weights were recorded and the animals then weighed every three days for fifteen days. Blood changes were recorded as follows: smears were taken every two days, beginning twenty four hours after the first injection of lead nitrate, and particular attention was paid to the presence of basophilic stippled red blood cells, polychromasia, and morphological changes in the r.b.c. The smears were stained according to May-Gruenwald-Giemsa and the above changes noted. For the recording of stippled cells, 30 fields of approximately 50 r.b.c. per field, were counted for the number of stippled cells and the total divided by 3, thus yielding the number of stippled cells per 500 r.b.c. (Tabel I-Code).

Following exitus, the animals were autopsied to rule out intestinal perforation and peritonitis as the cause of death.

Experimental Results

The animals were observed for a period of 4 weeks and the results recorded (Table I).

Group I: All the control animals died in a period of 1 to 21 days following the initial lead injection; the average date of exitus was 9 days. In this group, 75% of the animals died before the 13th day. The results are presented graphically in Table II and IV. Loss of weight and appetite were clearly apparent in 17 animals. Three of the control animals died within 3 days and it was thus too early to judge weight and appetite changes here. The average weight loss per animal after a period of 15 days was 81 grams, yielding an average weight loss per animal per day of 5.4 grams. These weight changes are graphically recorded in Table III.

Positive blood changes were not observed in any animal until the 5th day of experimental poisoning. All animals in this group surviving longer than 5 days were found to have positive blood pictures, with basophilic punctuated r.b.c., polychromatic cells, and anisocytotic and poikilocytic changes, ranging in degree from + to +++ (see Code, Table I.)

TABLE I: Group I: Control animals. Group II: Animals treated with BAL
Group III: Animals treated with sodium citrate

Group	Loss of weight	Loss of appetite	Red Blood-Cell changes			Exitus
			Stippled Cells	Polychromasia	Aniso.-Poikiloc.	
I:	1.	++	+	—	—	4 day
	2.	+	+	—	+	3 day
	3.	—	+	—	—	2 day
	4.	—	—	—	—	1 day
	5.	++	+	+	+	21 day
	6.	—	+	—	—	2 day
	7.	+	+	+	+	13 day
	8.	++	+	++	++	9 day
	9.	++	+	+	+	9 day
	10.	++	+	++	++	13 day
	11.	+	+	+	+	10 day
	12.	+	++	++	+	14 day
	13.	++	+	+	+	12 day
	14.	+	+	+	+	13 day
	15.	+	+	++	++	5 day
	16.	+	—	+	—	6 day
	17.	++	+	+	—	9 day
	18.	++	++	++	++	10 day
	19.	++	++	++	+	9 day
	20.	+	+	+	+	11 day
II:	1.	+	+	—	—	2 day
	2.	+	—	+	+	15 day
	3.	+	+	—	—	9 day
	4.	+	+	—	—	5 day
	5.	+	+	—	—	3 day
	6.	+	+	—	—	3 day
	7.	+	—	—	—	4 day
	8.	+	—	—	—	4 day
	9.	++	+	+	+	9 day
	10.	+	—	—	—	2 day
	11.	++	+	+	+	7 day
	12.	+	++	++	++	10 day
	13.	+	+	+	+	7 day
	14.	++	+	+	+	10 day
	15.	+	—	—	—	5 day†
	16.	—	—	—	—	2 day
	17.	+	+	+	+	12 day
	18.	+	—	—	—	4 day
	19.	—	++	++	++	15 day
	20.	—	—	—	—	2 day
III:	1.	+	—	—	—	10 day
	2.	—	—	—	—	—
	3.	+	+	—	—	10 day
	4.	—	++	++	++	—
	5.	+	+	—	—	10 day
	6.	—	+	+	—	—
	7.	+	—	+	—	11 day
	8.	+	—	—	—	3 day*
	9.	—	+	+	+	27 day
	10.	+	+	++	+	13 day
	11.	—	—	—	—	5 day
	12.	—	—	—	—	5 day
	13.	—	—	—	—	1 day*
	14.	—	+	+	+	20 day
	15.	+	—	—	—	9 day
	16.	+	+	—	—	13 day
	17.	—	—	++	+	—
	18.	—	++	++	++	16 day
	19.	—	+	++	+	24 day
	20.	+	+	—	—	12 day

Code: Loss of weight: (—) none, (+) 0-100 grams, (++) > 100 grams Code: Stippled cells; (—) none found.
(+) 1-2 per 500 R. B. C., (++) 3-6 per 500 R. B. C., (+++) 10 per 500 R. B. C., Morphological changes: (—) none,
(+) pale, slight aniso-, poikilocytosis, (++) slight aniso-, poikiloc. plus 1-3 nucleated R. B. C., (+++) above changes
plus many nucleated R. B. C.

Several animals showed signs of muscular paralyses and incoordination. These latter symptoms were not used as experimental criteria because of the difficulty in objectively interpreting them.

Group II: All 20 animals treated with BAL died in a period ranging from 2 to 15 days; the average date of exitus was 6.5 days. 90% of the animals died before the 13th day. Here again the animals showed significant losses in weight and appetite. The average loss of weight per animal after a period of 15 days was 89 grams, giving an average loss of weight per animal per day of 5.9 grams. Positive blood changes first appeared in this group 5 days after the initial lead injection and all animals surviving longer than this period had positive blood pictures. One animal (Table I†) had convulsive seizures following an injection of BAL and died soon after.

Group III: Of the 20 animals treated with sodium citrate, 4 animals were still alive 40 days after the initial lead injection and 16 died in a period ranging from 1 to 27 days; the average date of exitus here was 11.5 days. Two animals (Table I*) collapsed after the introduction of the sodium citrate through the stomach catheter and died soon after. Of the 16 animals, 50% died before the 13th day. Negative weight and appetite changes were recorded in half of the animals in this group. The average loss of weight per animal after 15 days was 24 grams or an average loss of weight per animal per day of 1.2 grams. The 4 surviving animals did not have any change in weight after 15 days and showed an average gain in weight after 30 days of 20 grams per animal.

Positive changes in the blood picture were not apparent till after the 10th day in this group, and of the 12 animals surviving longer than this period, 2 did not show any positive blood changes at all. 50% of the animals in this group, surviving longer than 6 days, did not show any positive blood changes (Group I: 7%, Group II: 11%).

Autopsies performed on the dead animals in all three groups did not reveal any evidence of perforation or peritonitis.^{4,5}

TABLE II:

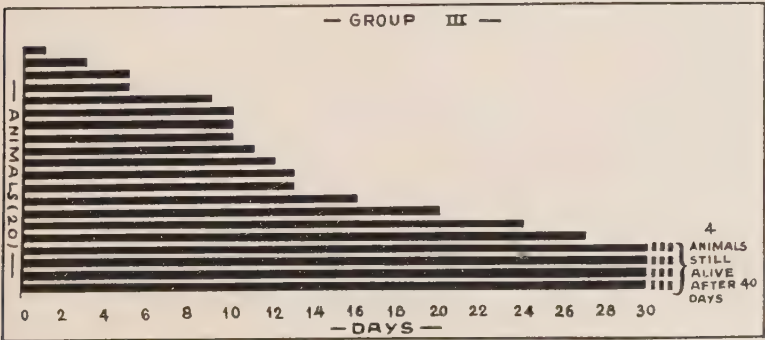
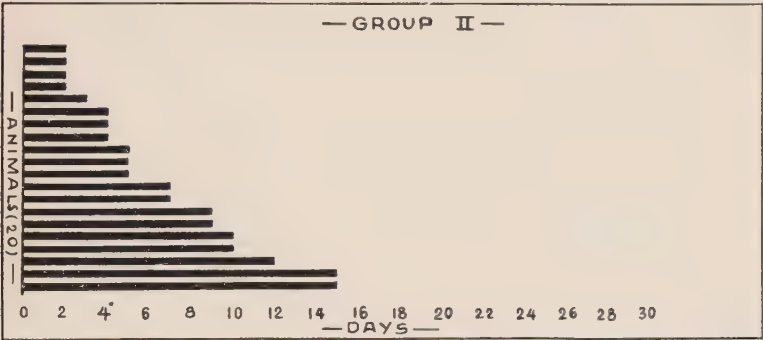
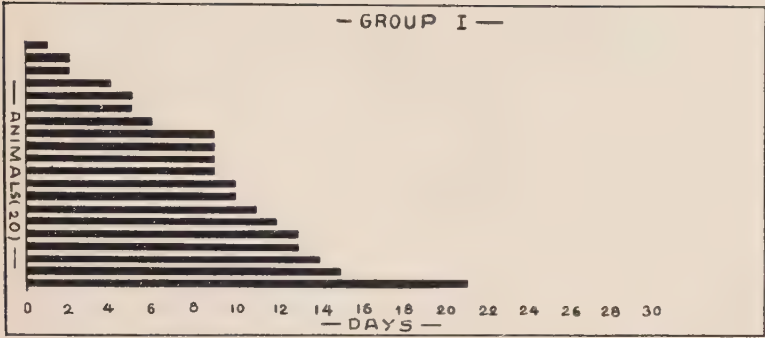


TABLE III

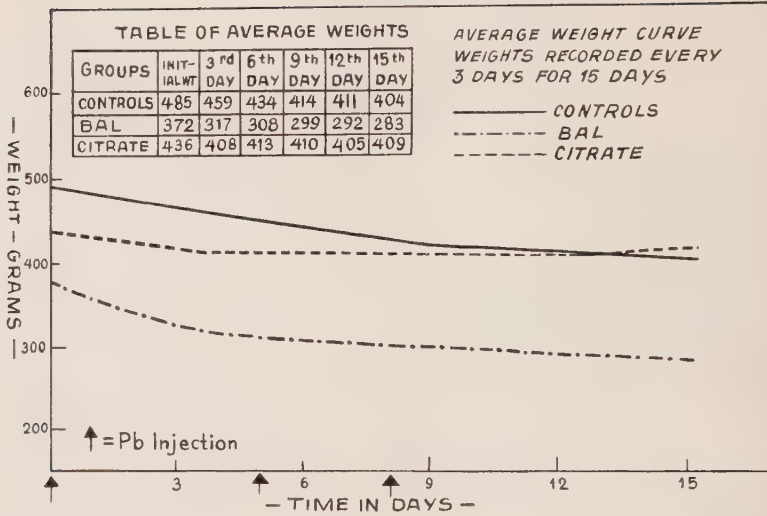
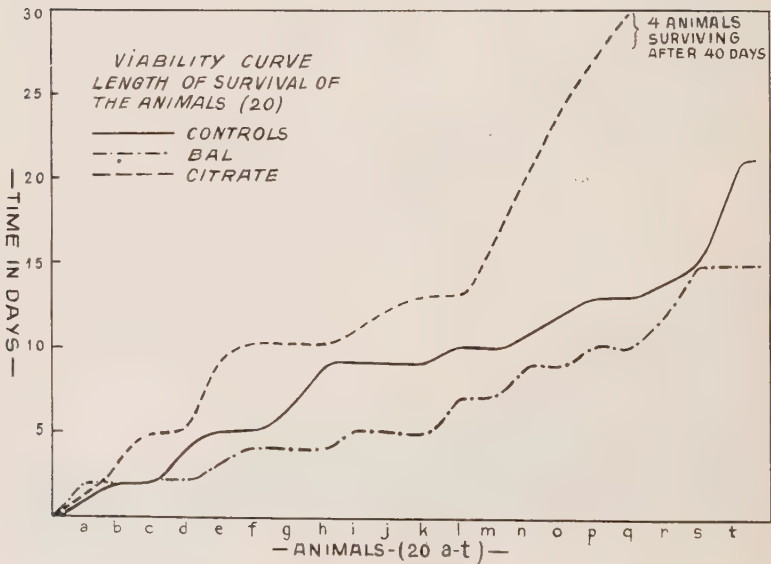


TABLE IV



III. DISCUSSION AND CONCLUSIONS

In 1940, Kety (1) demonstrated that dilute sodium citrate solution exerts a powerful solvent effect on the extremely insoluble tertiary lead phosphate. Subsequent investigations demonstrated that citrate forms with lead a soluble complex of extremely low dissociation. It was suggested further that the citrate ion normally present in human blood might have an important function in the transportation and excretion of lead. Mortensen (2), in a series of experiments seeking to explain the mechanism of citrate action, concluded that the presence of added citrate in the blood increases the percentage of lead taken up by the cells (in conflict with the results obtained by Bambach, Kehoe, and Logan (3) in similar studies); further, the addition of citrate greatly increases the ability of cells to combine with lead previously mixed with plasma; and finally, added citrate reduces the proportion of nondiffusible lead in plasma and serum. In a series of experiments, Kety (4) demonstrated that the administration of sodium citrate to lead poisoned rats, produced a significant increase in excretion of lead. A preliminary study (1) was undertaken clinically with 6 cases of lead poisoning. A rapid amelioration of symptoms, concurrent with an increase in lead urine excretion and a decrease in blood lead concentration, were observed after administration of sodium citrate. Clinical studies were made by Kety and Letonoff (5, 6) in 1943 on patients with chronic lead poisoning and striking results were obtained in a series of 15 patients with citrate therapy: An immediate and persistent amelioration of symptoms of lead poisoning during and after citrate therapy; a marked and significant fall in blood lead concentration and a significant increase in urinary and fecal lead excretion. They were further able to demonstrate that oral citrate produces an increase in blood citrate, which in turn brings about an increase in diffusible blood lead at the expense of non-diffusible lead of blood and eventually of the tissues. Furthermore it could be shown that during this process, the lead ion concentration of the blood cannot increase, thus the possibility of a rapid clearing of the blood lead and the ultimate extraction of the stored lead from the tissues should occur!

Our experimental results also indicate the value of sodium citrate in lead poisoning. While the results are not striking when viewed from the aspect of percentage survival in the citrate group (only 20% of the animals survived), it must be considered however, that extremely lethal doses of lead were administered to the animals (240 mgr./kgr. lead nitrate), through the intra-peritoneal injection route, a method of administration which produces a rapid and high concentration of systemic lead. In Tables III and IV however, in which the length of survival of the animals is presented graphically, a significant difference in viability between the citrate and control groups is demonstrated. In addition,

observations as to weight, appetite, and blood picture changes in this experiment, serve to confirm and amplify the significant tendency shown in the viability curves. For example, the average loss of weight per animal per day of 1.2 grams in the citrate group is strikingly lower than the corresponding values of 5.4 grams in the controls and 5.9 in the BAL treated animals. It is of interest to note, that the r.b.c. changes characteristic of lead poisoning, first appeared in the citrate group approximately twice as late as those in the control group. In several citrate animals, positive blood changes did not appear at all or very insignificantly. These observations would indicate a prophylactic value for sodium citrate, for example in lead exposed workers. There was not any correlation found between severity of symptoms and viability of the animals and the degree of characteristic red blood cell changes.

Of interest is an experiment by Donnelly and Holman (7), in which dogs were experimentally poisoned with uranium nitrate, resulting in kidney injuries to the animals. Use of sodium citrate facilitated repair and regeneration of the injured tubular epithelium, and resulted in the recovery of 92% of the animals receiving a lethal dose of uranium nitrate. Uranium is a metal similar in many ways to the action of lead in the body.

Our experiments indicate that BAL is not only ineffective in the treatment of sub-acute lead poisoning, but that its application is actually contraindicated. A comparison of the viability curves and weight figures of the BAL group with those of the control group, indicate that the BAL treated animals fared worse than the controls. This would confirm the results obtained by Braun et al (8), and Germuth and Eagle (9), in their experiments with BAL in the treatment of experimental lead poisoning. Braun found that the action of BAL was additive in rabbits acutely and chronically poisoned with lead. Germuth and Eagle similarly observed in their experiments, that BAL increases lead toxicity in many cases and that the increase in urinary lead excretion to each additional BAL injection vanished.

BAL, or 2,3-dimercaptopropanol was developed by Peters as an antidote to the arsenical «war gas» Lewisite (10). The toxicity of lead like many other heavy metals is probably due to interference with certain essential enzyme systems, in particular the sulfhydryl groups of the protein components of certain enzymes. This enzymatic inactivation is reversible by the sulfhydryl containing compound BAL. The success of BAL in the treatment of arsenic poisoning is attributed to the formation of a non-toxic, stable arsenic mercaptan, which is excretable by the kidney. In the case of lead, however, it appears that the lead-BAL complex is nearly or even more toxic than certain inorganic lead salts. It has been suggested by Lewis (11), in respect to this problem, that

while inorganic lead is deposited in the skeletal system, organic lead compounds (tetra ethyl i.e.) tend to attack the nervous system with greater avidity. In addition, as cited above, studies have shown, that the increase in lead excretion produced by BAL is transient, and tends to decrease with each additional injection of BAL. These observations would serve to explain the unfavorable action of BAL in lead poisoning.

Clinical trials with BAL have also proved disappointing. Telfer (12), reporting on the first case of lead poisoning (chronic) treated with BAL, found neither clinical improvement in the patient, nor toxic side effects, but suggested that BAL might be of use in the diagnosis of plumbism, since it raises urine lead to a confirmatory level. Telfer also suggested the possible use of BAL in the deleading process. Ryder and Kehoe (13) studied cases of acute lead poisoning treated with BAL and did not find it of any value, although the characteristic increases in urine lead were noted. These excretory increases were transient. The authors suggested that the failure of clinical response may be due to the possibility that the particular enzyme system affected by BAL is not involved in the mechanism of lead poisoning. Bastrup-Madsen (14, 15) did not find any amelioration of symptoms in two cases of acute lead poisoning treated with BAL, although mobilization of lead did not seem to aggravate the prevailing symptoms. Sundin (16) treated a case of chronic lead poisoning with BAL, sodium dihydrogen phosphate, and A.T. 10, and found the clinical condition distinctly improved.

The general principles in the therapy of lead poisoning have been stated by Cantarow (17) as follows:

- 1) Measures designed to limit absorption of lead from the intestine.
- 2) Measures designed to diminish the quantity of lead in the blood stream and tissue fluids by facilitating its deposition and storage in the skeleton.

- 3) After subsidence of manifestations of acute plumbism, measures designed to effect gradual mobilization of lead deposits and elimination of liberated lead from the body.

To limit intestinal absorption of lead (Point 1), magnesium sulfate lavage has been used with success. Moeschlin and Demiral (18) have demonstrated experimentally and clinically the efficacy of «Antidotum Metallorum Sauter», a stabilized H_2S solution, in acute thallium poisoning, another member of the heavy metal group. The action of the H_2S is of course the precipitation of the heavy metal as the insoluble sulfide, thus preventing its absorption. The H_2S preparation should be applied for a protracted period of time because of the reabsorption of the lead to some extent.

Under point 2, the high calcium and phosphorus intake therapy as formulated by Aub (19, 20) has been dominant for many years. This method of treatment is based theoretically on the parallel metabolism of

calcium and lead; thus a high calcium and phosphorus diet facilitates the deposition of lead in the bones. Sodium citrate, however, appears to provide a more effective means of treatment. Cantarow and Trumper (17) found that in their experience the most satisfactory results are obtained by the use of sodium citrate in addition to high calcium and phosphorus intake. Moeschlin (21) similarly has found sodium citrate the most effective therapy for lead poisoning as does Kornblitt (22) in his review of the cases of plumbism at the Zürich Clinic. The clinical trials of sodium citrate by Kety and Letonoff (1, 6) have already been noted. In their clinical studies, sodium citrate was used exclusively on the patients, with striking amelioration of all symptoms of chronic lead poisoning with the exception of constipation. Severe colic was relieved immediately by the intravenous administration of a sodium citrate solution.

Point 3 has been the subject of considerable controversy. Aub (19, 20) has suggested from a theoretical standpoint, that it would seem advantageous to remove stored lead in order to obviate the possibility of its sudden liberation during periods of metabolic stress. This «deleading process» is accomplished by means of a low calcium, high phosphorus diet, various acidifying agents (ammonium chloride i. e.); and such preparations as A. T. 10 and potassium iodide. The advisability of the «deleading process» has been questioned by many investigators, because of the possible recurrence of acute symptoms, following liberation of stored lead into the blood stream. Today, however, sodium citrate appears to be the drug of choice in plumbism, and would seem to obviate the necessity of a «deleading process». By virtue of its chemical action in plumbism, sodium citrate not only eventually liberates stored lead, but reduces the freed lead in the blood stream to a non-ionized, innocuous, soluble, stable, easily excretable citrate salt. For an aggressive therapy in chronic lead poisoning a combination of both sodium citrate and low calcium diet would seem advisable.

On the basis of our experimental and clinical experiences and the various studies discussed above, we would recommend the following procedures for the treatment of lead poisoning:

I. Acute Poisoning

1. Do not use BAL.
2. Gastric lavage, with the addition of 30 gr. of powdered charcoal, providing the ingestion of the poison has taken place within 12 hours.
3. «Antidotum Sauter»: Give 100 ccm. stabilized H_2S solution through the stomach tube. The H_2S sometimes produces light collapse

symptoms, which can be controlled through the application of analeptics.

4. Start with citrate therapy immediately. For adults, give 4 or 5 grams of sodium citrate dissolved in 30 ccm, (1 ounce) of water, 3—4 times daily. Children receive 1—2 grams, 3 times a day.
5. Colic: 20 ccm. of a 20% calcium gluconate solution, i. v., usually gives relief. In addition local heat, anti-spasmodics, and sedatives. 50 ccm. of a sterile 2.5% aqueous solution of sodium citrate has given prompt relief to severe colic.

II. Chronic Poisoning

1. Remove permanently from exposure.
2. Low calcium, high phosphorus diet (Ca: P, 1:4).
3. Treat with sodium citrate as above.
4. Treatment of constipation to avoid reabsorption of lead.
5. Palsy should be treated with galvanic currents and massage.

IV. SUMMARY

1. A study was made to compare the efficacy of BAL and sodium citrate in the treatment of (sub-acute) experimental lead poisoning in guinea pigs.
2. The experimental results indicate that BAL is not only ineffective in the treatment of guinea pigs sub-acutely poisoned with lead (lead nitrate solution), but that it increases lead toxicity. Sodium citrate on the other hand, revealed itself as an effective therapeutic measure in this experiment. These conclusions were based on the following criteria: Viability curve, characteristic red blood cell changes, and variations in weight and appetite of the animals.
3. The experimental results and other clinical and experimental studies were discussed.
4. On this basis, recommendations were made for the treatment of acute and chronic lead poisoning.

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CURRICULUM VITAE

I was born in Antwerp, Belgium on July 15, 1928, and migrated with my family to America at the age of one. I studied at the Stuyvesant High School of New York and at the New York University, from which institution I received my A. B. degree in June, 1948. I commenced my medical studies at the Faculty of Medicine of the University of Zürich in 1948 and completed the course in 1953.

